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Dolphin Morbillivirus Infection in a Captive Harbor Seal (*Phoca vitulina*)

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During the second morbillivirus epidemic (2007 to 2011) in cetaceans along the Italian coastline, dolphin morbillivirus (DMV) was detected by molecular analyses in a captive harbor seal (*Phoca vitulina*), with pathological findings consistent with morbillivirus infection. This report confirms interspecies DMV transmission from cetaceans to pinnipeds.

CASE REPORT

During the second dolphin morbillivirus (DMV) epidemic in the Mediterranean Sea (2006 to 2011), several cetaceans stranded on the Tyrrhenian coast of Italy were recovered by the national stranding network between 2010 and 2011. In the summer of 2011, one live bottlenose dolphin (*Tursiops truncatus*) (no. 48258; Istituto Zooprofilattico Sperimentale del Piemonte, della Liguria e della Val d'Aosta [IZSPLVA], Turin, Italy) and one dead striped dolphin (*Stenella coeruleoalba*) (no. 48203; IZSPLVA) tested positive for morbillivirus by reverse transcription (RT)-PCR only in the brain, without immunohistochemical evidence of morbillivirus antigen in any other tissue, as reported elsewhere (1). The field operations had been supported by personnel of a zoo during rehabilitation of the live bottlenose dolphin, which died after 1 day of medical care and life support. These personnel were not usually involved in zoological activities and had not entered the zoo for 1 week or animal facilities for 3 weeks after either stranding event. Furthermore, to prevent disease transmission to the zoo animals, disposable material was regularly used and incinerated as medical wastes, with durable equipment systematically disinfected by means of a commercial mixture based on ethyl-isopropyl alcohol, quaternary ammonium salts, and glutaraldehyde and kept external to the zoo enclosures. Despite these precautions, 28 days after the bottlenose dolphin stranding event, an adult male harbor seal (*Phoca vitulina*) (no. 204; University of Padua, Padua, Italy) hosted inside the zoo died shortly after developing anorexia, tremors, abdominal contractions, and polyuria, with hypothermia and vomiting just before death.

A detailed necropsy was carried out within 24 h after death; tissue samples were preserved in 10% neutral buffered formalin for histopathology, refrigerated for microbiology and parasitology, and frozen for biomolecular investigations.

Postmortem examination showed extensive, multifocal to coalescent colliquative renal necrosis and focal centrilobular liver necrosis, in addition to mild gastritis, moderate acute necrotic enteritis, hemorrhagic foci in the liver, spleen, and lungs, and severe mediastinal emphysema. Histopathological findings were consistent with gross alterations: necrotic lesions occasionally associated with mild hemorrhages and, rarely, with mild fibrinous effusions. Neutrophil and fibrin clusters were evident within the vascular lumina in all major organs, with hyperplasia/hypertrophy of pulmonary intravascular macrophages and splenic hemosiderophages. Gram-negative coccoid bacteria adherent to epithelial cells of intestinal glands and within the luminal contents were seen at microscopic examination. On microbiological investigation, *Aeromonas hydrophila* was isolated from the spleen and the intestinal tract and presumed responsible for the septic shock and subsequent death of the animal.

Besides the aforementioned pathological findings, generalized and diffuse lymph node enlargement and prominent meningeal hyperemia and choroid plexus edema were observed. Microscopically, severe, diffuse reactive lymphadenitis was noted, accompanied by lymphoid cell depletion and hyalinosis in germinal centers and several multinucleate syncytia (Fig. 1A). Also apparent were mild, multifocal, chronic choriomeningitis with rare mononuclear cell perivascular cuffs (Fig. 1B), and mild, multifocal white matter spongiosis and demyelination. Rare cytoplasmic eosinophilic inclusion bodies were found in glial cells (Fig. 1C). Similar cytoplasmic inclusions were seen in the epithelial cells lining the urinary bladder and palatine tonsils, along with moderate, multifocal lymphocytic inflammation and epithelial cell apoptosis. Mild, chronic bronchointerstitial pneumonia, with evidence of bronchiolar epithelial cell hyperplasia/hypertrophy, was also observed.

Pathological findings were suggestive of morbillivirus infection, which was subsequently confirmed by RT-PCR (2) with DMV-specific genome sequences amplified from the lungs, inguinal lymph nodes, and brain (3). Biomolecular investigation for canine distemper virus (CDV) and phocine distemper virus (PDV), performed according to the protocol described by Stanton et al. (4), was negative. Partial nucleoprotein (N1), fusion protein (F), and hemagglutinin (H) gene regions were amplified (Table 1) using cetacean morbillivirus (CeMV)-specific primers (5). The incongruence-length-difference (ILD) test (WinClada version

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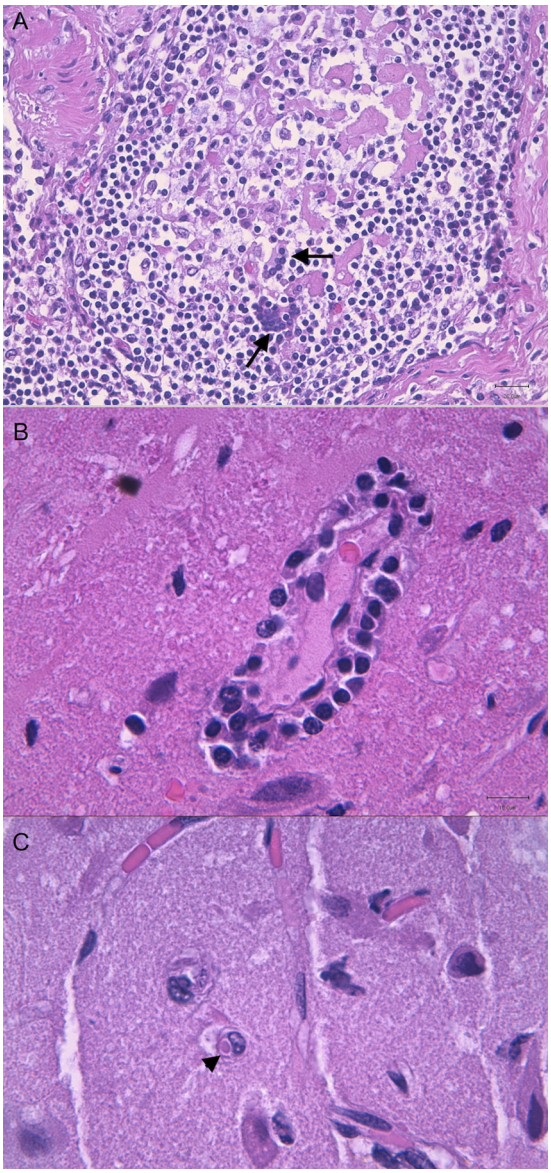

FIG 1 Microscopic findings in the harbor seal with DMV infection. Note the lymphoid cell depletion and multinucleate syncytia in the prescapular lymph nodes (arrows) (hematoxylin-eosin stain [HE]; magnification, ×10; bar, 20 μm) (A) and the mononuclear cell perivascular cuffing around a small capillary in the brain (HE; magnification, ×100; bar, 10 μm) (B), with an intracytoplasmic viral inclusion body inside a glial cell (arrowhead) (HE; magnification, ×100; bar, 10 μm) (C).

1.00.08) demonstrated that the three data sets were not significantly incongruent ($P = 0.2467$). Therefore, an approach combining the N1, F, and H gene regions was selected for phylogenetic analysis. The sequences were concatenated into a multigene alignment to increase the discriminatory power. Phylogeny inference according to the maximum likelihood criterion was performed using MEGA version 5. The nucleotide substitution model was general time reversible (GTR), with four substitution rate categories. The robustness of the hypothesis was tested in 1,000 nonparametric bootstrap analyses.

The resulting phylogenetic tree showed a well-separated cluster corresponding to the available DMV sequences (Fig. 2). Even though the analysis, conducted at the same time on the three animals, indicated that the overall genetic diversity of the known circulating DMV strains was low, which is consistent with previous findings (6), our multigene approach allowed us to discriminate a distinct subclade, supported by a 66% bootstrap value and including the DMV sequences obtained from the harbor seal and the two dolphins stranded in 2011. The sequences showed a 100% similarity among them but shared unique nucleotide differences compared to the DMV reference sequences, strongly supporting the hypothesis for DMV transmission from the stranded dolphins to the captive seal.

Over the last 25 years, morbillivirus infections have caused dramatic mortalities among aquatic mammal species and populations worldwide. DMV poses a major threat for free-ranging cetaceans. It has been responsible for two epidemics in the Mediterranean Sea (6): the first outbreak (1990 to 1992) affected striped dolphins (*Stenella coeruleoalba*) (7, 8), while the second (2006 to 2011) involved several other species besides striped dolphins, including pilot whales (*Globicephala melas*), bottlenose dolphins (*Tursiops truncatus*), Risso's dolphins (*Grampus griseus*), and fin whales (*Balaenoptera physalus*) (1, 8–11). Among pinnipeds, mortality events worldwide have involved harbor seals (*Phoca vitulina*), gray seals (*Halichoerus grypus*), Baikal seals (*Pusa sibirica*), and Caspian seals (*Pusa caspica*) by either CDV or PDV, an agent closely related to CDV (7).

Although members of the *Morbillivirus* genus identified in cetaceans and pinnipeds belong to separate phylogenetic clusters (12), a virus closely related, but not identical, to DMV was isolated from Mediterranean monk seals (*Monachus monachus*) found dead during an outbreak which had resulted in mass mortality off the coast of Mauritania (13). The tentative link between the mass die-off dolphins in the coastal waters of Mauritania and the preceding monk seal event in the same area suggested that interspecies transmission of the virus could occur between cetaceans and pinnipeds. However, the lack of specimens from the dead dolphins precluded the notion of interspecies viral transmission.

TABLE 1 Morbillivirus names and accession numbers

Morbillivirus name[a]	Accession no.[b]		
	N	F	H
DMV 2011 *Phoca vitulina* ID 204	HF570930	HF570933	HF570936
DMV 2011 *Tursiops truncatus* ID 48258	HF570931	HF570934	HF570937
DMV 2011 *Stenella coeruleoalba* ID 48203	HF570932	HF570935	HF570938
DMV Gme/2007	HQ829972	HQ829972	HQ829972
DMV_Sc/2007	HQ829973	HQ829973	HQ829973
DMV 2003	AJ608288	AJ608288	AJ608288
CeMV 20E	FJ842380	FJ842382	FJ842382
CDV isolate 164071	EU716337	EU716337	EU716337
PDV	X75717	D10371	D10371
PPRV	AJ849636	AJ849636	AJ849636
RPV	X98291	X98291	X98291
MeV	AB254456	AB254456	AB254456

[a] DMV, dolphin morbillivirus; CeMV, cetacean morbillivirus; CDV, canine distemper virus; PDV, phocine distemper virus; PPRV, peste-des-petits-ruminants virus; RPV, rinderpest virus; MeV, measles virus.
[b] The entries for the DMV 2011 isolates are EMBL accession numbers; all other entries are GenBank accession numbers.

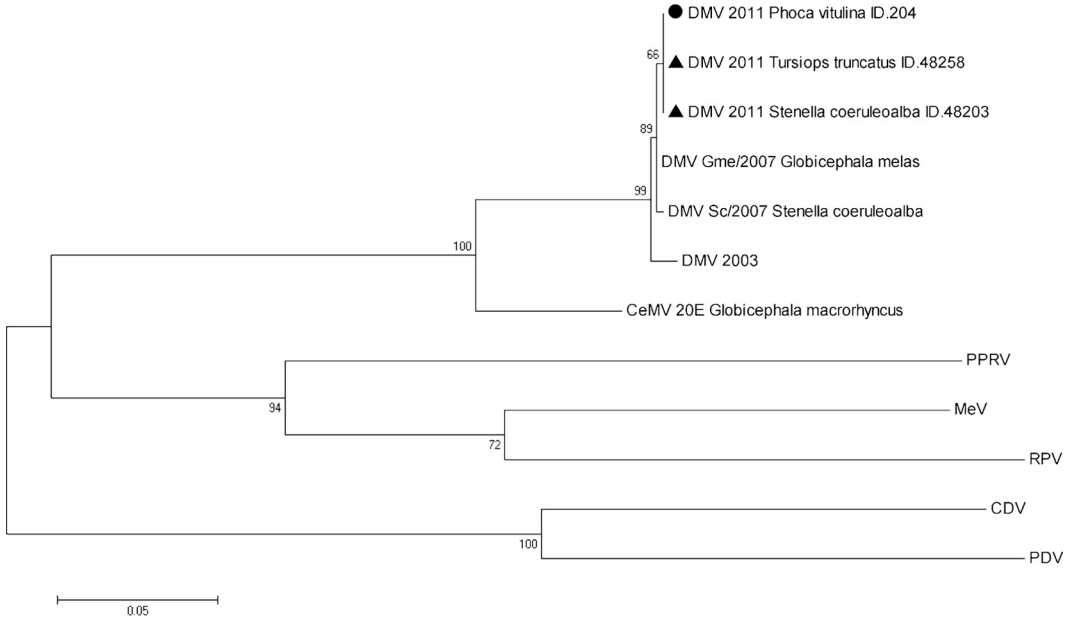

FIG 2 Phylogenetic tree. Morbillivirus phylogeny inferred by maximum likelihood (ML) analysis of the nucleoprotein (N), fusion protein (F), and hemagglutinin (H) genes combined in a single data set of 503 nucleotides. DMV sequences identified in this study are labeled (circle, harbor seal; triangles, stranded dolphins). DMV, dolphin morbillivirus; CeMV, cetacean morbillivirus; CDV, canine distemper virus; PDV, phocine distemper virus; PPRV, peste-des-petits-ruminants virus; RPV, rinderpest virus; MeV, measles virus. The infected species and the year of stranding or sequence submission for cetacean morbilliviruses are indicated, if available. Table 1 reports the accession numbers of the analyzed sequences. Bootstrap (1,000 replicates) values of >50 are indicated at the internal nodes. The length of each pair of branches represents the distance between the sequence pairs. The scale bar represents the percentage of nucleotide differences.

This report describes a spontaneous DMV infection that caused disease in a harbor seal, with epidemiological, molecular, phylogenetic, and pathological data strongly supporting the assumption that interspecies DMV transmission from cetaceans to pinnipeds may occur, as previously suggested for monk seals (13). That morbillivirus can switch from one host species to another has been already demonstrated for CDV infection. Indeed, the repeated independent emergence of CDV in novel species appears to be associated with its adaptation to receptor-binding regions determining virus-host specificity (14). Furthermore, throughout the 2006 to 2011 morbillivirus epidemic in the Mediterranean Sea, DMV infection was repeatedly reported in several species (1, 8–11). Another unprecedented finding of our study is that DMV may not only infect but also cause severe disease and subsequent mortality in harbor seals, without previous species adaptation, whereas DMV-like infection could not be established as the primary cause of mortality in the monk seals from Mauritania (7). Seropositivity to morbillivirus in captive marine mammals has been previously assessed, although an infectious source in the wild was suspected for these animals (15).

The two stranded cetaceans, particularly the live bottlenose dolphin, might be hypothesized as the presumptive source of DMV infection in the captive seal on the basis of the overlap of biomolecular characterization; however, the exact route by which the virus entered the zoo facility remains unknown. This uncertainty is additionally underscored by the rapid inactivation of *Paramyxoviridae* under normal environmental conditions (16). Nevertheless, morbillivirus infection has been reported among captive carnivores in zoological facilities, with a direct contact between CDV-infected and uninfected live animals considered a necessary means for viral transmission (17). In our case, however, no morbillivirus-positive wild animals were known to have ever entered the zoo, reasonably excluding the possibility of indirect infection from water or food contamination by aerosols, secretions, or excretions. Furthermore, the quarantine the zoo administrators had imposed following the two wild dolphin strandings reasonably excludes zoo staff or equipment as a potential source of viral contamination, although a role of indirect carriers cannot be completely ruled out due to common biologic and epidemiologic features of morbilliviruses, such as their high transmission rate and low minimum infectious dose (18).

Morbillivirus infections have long been known to be associated with increased immunosuppression in their natural hosts (19). DMV has been frequently reported in association with secondary infections from opportunistic bacteria or protozoa such as *Toxoplasma gondii* (1, 7, 8, 11). In seals, *Aeromonas* spp. has been suggested as a possible opportunistic pathogen in morbillivirus-infected individuals (20, 21); likewise, the *Aeromonas hydrophila* strain recovered from the spleen and the intestinal tract of our DMV-infected captive seal should be considered a secondary pathogen responsible for the acute septic shock and subsequent death in this animal. *Aeromonas* spp. have been frequently reported in human and veterinary medicine as a cause of acute gastroenteritis, wound infection, and septicemia due to the multiple virulence factors the bacteria produce (21).

In conclusion, the present case of DMV infection in a captive harbor seal, which occurred without direct contact with two DMV-infected dolphins, calls for new guidelines to enforce and extend quarantine protocols for zoological parks housing marine mammals and rehabilitating animals, considering the biologic features of the DMV isolates reported here and the potential for interspecies viral transmission (15, 17).

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We declare no competing interests.

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